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FIRST PERSON

First person – Alexander Johnson

First Person is a series of interviews with the first authors of a selection of papers published in Journal of Cell Science, helping early-career researchers promote themselves alongside their papers. Alexander Johnson is first author on 'Experimental toolbox for quantitative evaluation of clathrin-mediated endocytosis in the plant model *Arabidopsis*', published in JCS. Alexander is a postdoc in the lab of Jiri Friml at the Institute of Science and Technology Austria, investigating the characterization of the molecular mechanisms of endocytosis in plants using a range of quantitative imaging and biochemical approaches.

Alexander Johnson

How would you explain the main findings of your paper in lay terms?

Endocytosis is a key process in all cells as it mediates the entry of cargos into the cell. Given its importance, it is surprising that not much is known about how it actually works in plants. What we do know is that there are major differences in how plant endocytosis functions compared to how it works in mammalian and yeast systems. As plants have to overcome their unique physiological pressures, it is thus incredibly interesting to study their alternative endocytosis mechanisms. In order to aid the advancement of plant endocytosis characterization, we provide here a 'beginners guide to imaging plant endocytosis'. We focused on quantitative imaging methods as they allow the direct examination of the complex endocytosis process. We present methods which allow the characterization of plant endocytosis at three scales: (1) the ultrastructural view of endocytosis structures, (2) the live dynamics of single endocytosis events in cells and (3) the overall efficiency of endocytosis at the tissue level. With these, we provide the plant endocytosis community with a range of standardized tools to investigate many unique aspects of plant endocytosis.

["..we provide here a 'beginners guide to imaging plant endocytosis'"]

Were there any specific challenges associated with this project? If so, how did you overcome them?

Establishing a reliable method for TIRF-M for the intact plant samples was tough. For example, plants have a cell wall, which is optically very 'unfriendly' (it had even been suggested that TIRF would be impossible with plant samples), and roots are surprisingly soft making it very easy to destroy them during their preparation for imaging. Luckily, I have had the chance to interact with many experts in both microscopy and plant physiology, and we have been able to solve many of these issues.

When doing the research, did you have a particular result or 'eureka' moment that has stuck with you?

I think the biggest 'eureka'/breakthrough moment was the first time I ran some of the analysis code and it completed without an error! I didn't have much experience with coding and there is quite a lot of code presented in this paper. It is definitely a very different skill from wet lab research, but with a lot of trial and error, and many months of testing; it was a very good feeling to get it all working!

Why did you choose Journal of Cell Science for your paper?

The idea for this paper actually came from a Company of Biologists Workshop focused on plant endocytosis ('Cellular Gateways: Expanding the Role of Endocytosis in Plant Development'). During the discussion sessions it became clear that a set of standardized tools to quantify endocytosis investigations would be of benefit to the community, as it would allow the direct comparison of experiments from differing groups. Therefore, the Journal of Cell Science seemed like a very fitting choice.

Have you had any significant mentors who have helped you beyond supervision in the lab? How was their guidance special?

Yes! Firstly, my late friend and first postdoc mentor, Christien Merrifield. While I was a postdoc in his lab, he taught me the fundamentals and importance of imaging and analysis combined with technical innovations. He has shaped a lot of my beliefs about research, and his pursuit of experimental excellence is one quality I try and apply to everything I do. He was also key in proving that TIRF-M was indeed possible with plants. One of my favorite memories was the excitement of working together on his homemade TIRF setup, looking at plants together for the first time. Neither of us had any idea how, and if, it would work! It was truly explorative imaging, and luckily, we got it to work, and that was the start of my

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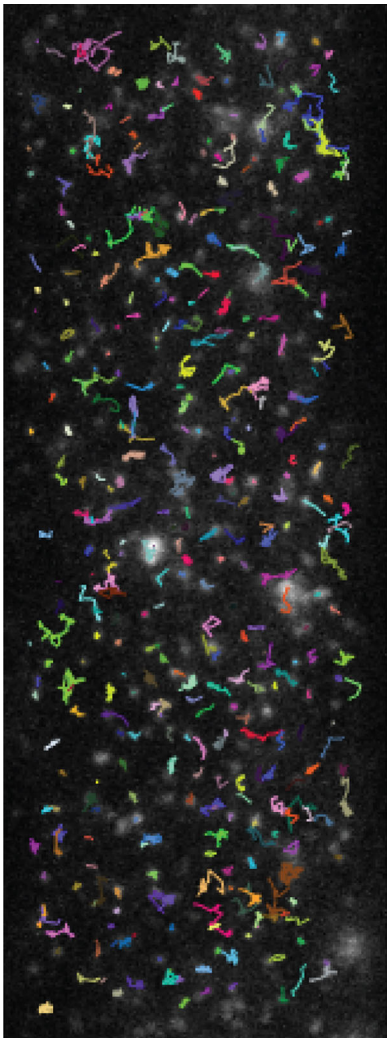

Tracking single events of clathrin-mediated endocytosis in an intact *Arabidopsis* root epidermal cell. The plant is expressing CLC2–GFP and is imaged using TIRF-M. The colorful trails represent the tracks of CLC2 foci automatically detected and tracked using the analysis system presented in this paper.

career in plants. This paper is dedicated to his memory, and I am very honored to have learnt from him.

I would also like to mention Gregory Vert (who was my first plant supervisor and trusted that we could get these approaches to work with plant samples), Sebastian Bednarek (for his plentiful and friendly advice) and finally Jiri Friml (for supporting and aiding in the refinement and development of these methods).

What motivated you to pursue a career in science, and what have been the most interesting moments on the path that led you to where you are now?

I really enjoy finding out how things work, so science was always an easy choice for me. I particularly enjoy the challenge of figuring out 'how to find out how things work', so relish learning about different methods and techniques that I can try to apply to my own specific research questions. One of the most interesting things for me has been the transfer and optimization of the experimental techniques that I learnt in mammalian cells to plants.

Who are your role models in science? Why?

There are lots as I believe we can learn something from everyone. With everyone, I try to identify their positive traits and get ideas to incorporate into my own style. It means I can also learn from 'less positive' experiences, which is just as important as learning from great role models.

What's next for you?

I hope to find a research position where I can continue to work with plants, specifically to develop imaging approaches to further investigate the molecular mechanisms of plant endocytosis and how it differs from other model systems.

Tell us something interesting about yourself that wouldn't be on your CV

I love outdoor sports and used to be an avid alpinist. But these days, with lab work and children, I don't get out so much!

Reference

Johnson, A., Gnyliukh, N., Kaufmann, W. A., Narasimhan, M., Vert, G., Bednarek, S. Y. and Friml, J. (2020). Experimental toolbox for quantitative evaluation of clathrin-mediated endocytosis in the plant model *Arabidopsis*. *J. Cell Sci.* **133**, jcs248062. doi:10.1242/jcs.248062